

WHAT IS CLAIMED IS:

1. An (R)-2-octanol dehydrogenase having the following physicochemical properties (1) and (2):

(1) Action

5 i) The enzyme produces ketone by oxidizing alcohol using oxidized form of β -nicotinamide adenine dinucleotide as a coenzyme, and

10 ii) The enzyme produces alcohol by reducing ketone using reduced form of β -nicotinamide adenine dinucleotide as a coenzyme, and

(2) Substrate specificity

15 i) The enzyme preferentially oxidizes (R)-2-octanol of two optical isomers of 2-octanol, and

15 ii) The enzyme produces (S)-4-halo-3-hydroxybutyric acid esters by reducing 4-haloacetoacetic acid esters.

2. The (R)-2-octanol dehydrogenase of claim 1 having the following physicochemical properties (3) and (4):

(3) Optimum pH

20 Optimum pH for the oxidation reaction ranges from 8.0 to 11.0, and that for the reduction ranges from 5.0 to 6.5, and

(4) Substrate specificity

i) The enzyme shows higher activity on secondary alcohols than on primary alcohols, and

25 ii) The enzyme shows significantly higher activity on (R)-2-octanol than on 2-propanol.

3. The (R)-2-octanol dehydrogenase of claim 1, wherein the (R)-2-octanol dehydrogenase is derived from a microorganism selected from the group consisting of the genus *Pichia*, genus *Candida*, and genus *Ogataea*.

4. The (R)-2-octanol dehydrogenase of claim 3, wherein the microorganism belonging to the genus *Pichia* is *Pichia finlandica*.

5 5. The (R)-2-octanol dehydrogenase of claim 3, wherein the microorganism belonging to the genus *Pichia* is *Pichia jadinii*.

6. The (R)-2-octanol dehydrogenase of claim 3, wherein the microorganism belonging to the genus *Candida* is *Candida utilis*.

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7. The (R)-2-octanol dehydrogenase of claim 3, wherein the microorganism belonging to the genus *Ogataea* is *Ogataea wickerhamii*.

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8. A method for producing the (R)-2-octanol dehydrogenase of claim 1, the method comprising cultivating a microorganism selected from the group consisting of the genus *Pichia*, genus *Candida*, and the genus *Ogataea*, the microorganism producing the enzyme of claim 1.

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9. An isolated polynucleotide of (a) to (e) below, the polynucleotide encoding a protein having activity of (R)-2-octanol dehydrogenase:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1,
- (b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2,
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2 in which one or more amino acids are replaced, deleted, inserted, and/or added,
- (d) a polynucleotide hybridizing under stringent conditions with a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1, and
- (e) a polynucleotide encoding an amino acid sequence having not less than 70% homology to the amino acid sequence of SEQ ID NO:2.

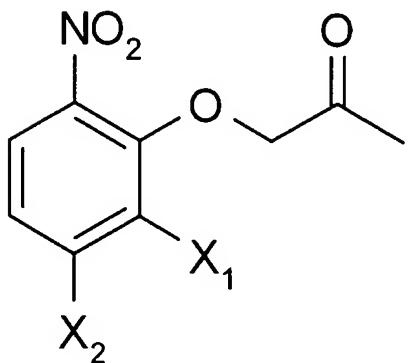
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10. A substantially pure protein encoded by the polynucleotide of claim 9.
11. A recombinant vector into which the polynucleotide of claim 9 is inserted.
- 5 12. The recombinant vector of claim 11, wherein a polynucleotide encoding a dehydrogenase that can catalyze oxidation-reduction reaction using β -nicotinamide adenine dinucleotide as a coenzyme is further inserted.
13. A transformant comprising the vector of claim 11 in an expressible manner.
- 10 14. A method for producing a protein, the method comprising cultivating the transformant of claim 13.
- 15 15. A method for producing an alcohol, the method comprising reacting the (R)-2-octanol dehydrogenase of claim 1, a microorganism producing the enzyme or the protein, or a processed product of the microorganism with a ketone to reduce the ketone.
- 20 16. The method of claim 15, wherein the microorganism is a transformant comprising a recombinant vector into which a polynucleotide encoding a (R)-2-octanol dehydrogenase is inserted.
17. The method of claim 15, wherein the ketone is a 4-haloacetoacetic acid ester derivative and wherein the alcohol is an (S)-4-halo-3-hydroxybutyric acid ester derivative.
- 25 18. The method of claim 17, wherein the 4-haloacetoacetic acid ester derivative is 4-chloroacetoacetic acid ethyl ester and wherein the alcohol is (S)-4-chloro-3-hydroxybutyric acid ethyl ester.

19. The method of claim 15, wherein the ketone is an acetyloxybenzene derivative represented by the generic formula 1:

generic formula 1

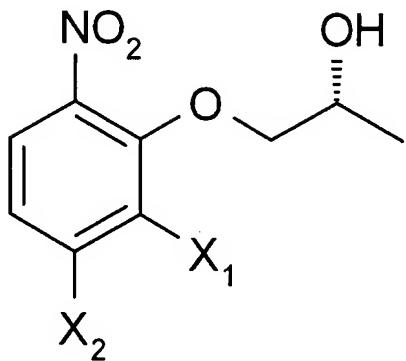
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where each of $\text{x}1$ and $\text{x}2$ indicates a halogen atom; and wherein the alcohol is a propoxybenzene derivative represented by the generic formula 2:

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generic formula 2



20. The method of claim 19, wherein the acetyloxybenzene derivative is
15 2-acetyloxy-3,4-difluoronitrobenzene and wherein the alcohol is 2,3-difluoro-6-nitro[[(R) -2-hydroxypropyl]oxy]benzene.

21. The method of claim 15, the method further comprising converting oxidized form of β -nicotinamide adenine dinucleotide into reduced form thereof.

5 22. A method for producing a ketone, the method comprising reacting the (R)-2-octanol dehydrogenase of claim 1, a microorganism producing the enzyme or the protein, or a processed product of the microorganism with an alcohol to oxidize the alcohol.

10 23. A method for producing an optically active alcohol, the method comprising the steps of reacting the (R)-2-octanol dehydrogenase of claim 1, a microorganism producing the enzymes or the protein, or a processed product of the microorganism with a racemic alcohol to preferentially oxidize either optical isomer, and obtaining the remaining optically active alcohol.

15 24. The method of claim 22, the method further comprising converting reduced form of β -nicotinamide adenine dinucleotide into oxidized form thereof.